Work plan and methodology

- **Collection of plant materials.**
  - Collection of plant materials whole plant, aerial part and tissues (leaves, stem, barks) from different locations.
  - Plants/parts which are free from diseases and microbial infection will be collected.

- **Optimization of extraction from dried plant materials.**
  - The solvent extraction has been the most common method for the extraction of diverse compounds found in plants. However, the mode of extraction depends on knowledge of physicochemical properties of compound that is being studied including:
    - partition coefficients in water or organic solvents
    - relative polarity of the molecule
    - stability of molecule in light or dark
    - temperature employed during extraction
  - Usually, green techniques of extraction such as ultrasound assisted extraction, microwave assisted extraction and super critical fluid extraction, enzymatic extraction have been widely utilize for phytochemical processing of medicinal plants.

- **Preliminary phytochemical analysis**
  - Plants consist of number of biologically active ingredients such as alkaloids, flavonoids, glycosides, terpenes, tannins and phenolic compounds.
  - Each group has particular qualitative method of analysis which varies on plant to plant, e. g. the reactions of phenols with the Folin-Denis and the

Folin-Ciocalteu reagents have been found to be stoichiometrically predictable.

- **Characterization of major phenolic compounds using spectroscopic and chromatographic techniques.**
  - The conventional extraction methods are not selective for major phenolics; since they are able to co- extract the great number of other high molecular weight compounds. So it is necessary to implement new technique to achieve selective extraction.
  - In these sense, it has been proposed a wide variety of techniques; from extractions in solid phase (SPE) and liquid- liquid (LLE) up to the use of sophisticated chromatography techniques like counter current chromatography (CCC), medium pressure liquid chromatography (MPLC) and the high performance liquid chromatography (HPLC)
  - For characterization, HPLC with photo diode array detection has been used but the main drawback of this technique is the difficulty to obtain reference compounds and the spectral similarities. Therefore, mass spectrometry (MS) and nuclear magnetic resonance (NMR) of ^{1}H and ^{13}C have become the preferred techniques for identification and characterization.

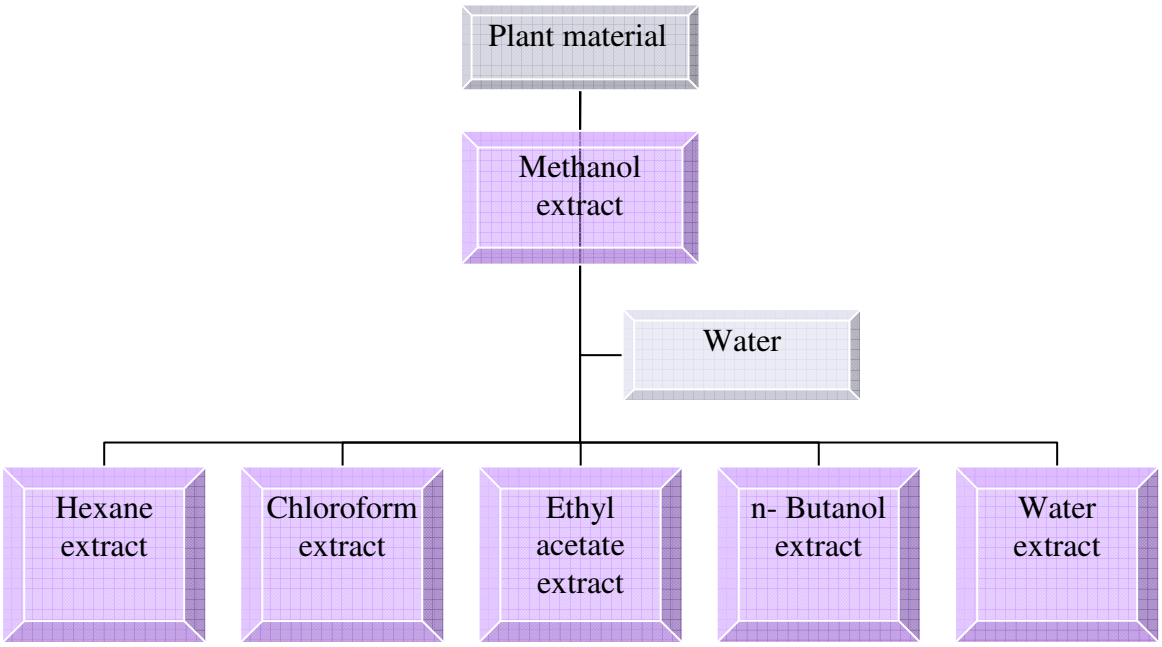
- **Development of HPLC method and its validation for identification and quantification of marker phenolics.**
  - Thin layer chromatography (TLC), gas chromatography (GC), HPLC, electrophoretic methods, spectrophotometric detection, etc. has been widely utilized for phenolic analysis in natural plants
  - In last few years, HPLC is become as an analytical technique that has dominated the separation and characterization of phenolic compounds. HPLC techniques offer a unique chance to separate simultaneously all

analyses components together with their derivatives and degradation products.

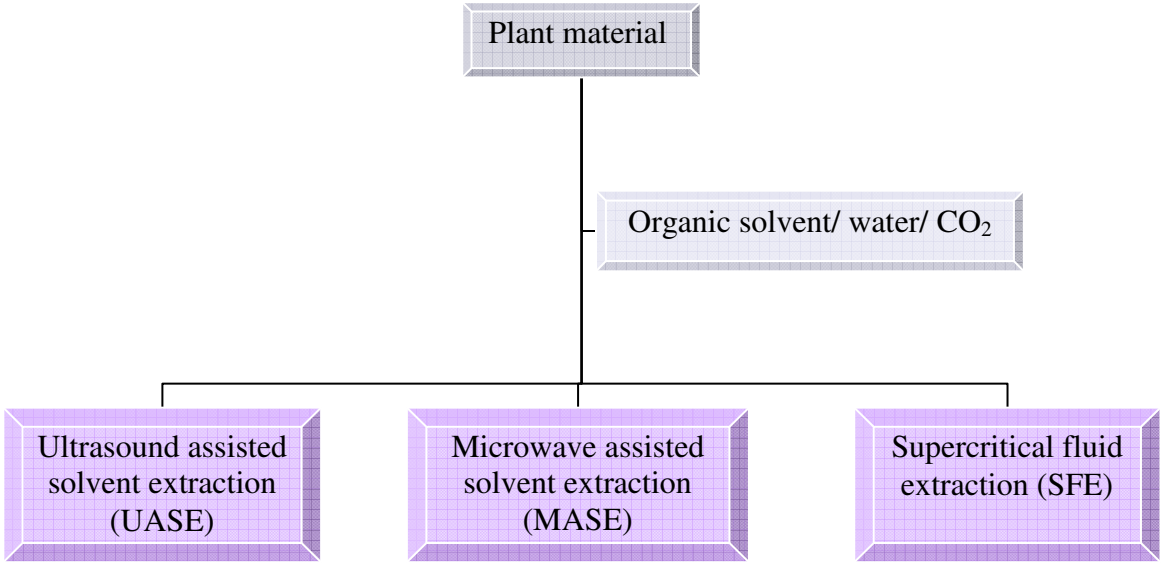
- There are many advantages dictating the wide spread use of HPLC in the analysis of phenolic compounds in plant- derived and biological matrices, such as,
  - The wide range of commercially available columns, including those using new generation sorbents with fit-for- purpose properties.
  - The possibility of combining two or more column in a switching mode.

Anti- oxidant assay of different extracts and individual phytochemicals.

- The compounds which are easier to oxidize are often the best oxidants. Several studies have suggested that the phenolic content and their corresponding antioxidant activity, contributes to the fruits and vegetables protective effect against degenerative and chronic disease. Some plant and fruits with high phenolic compounds content have been reported to act as mutagenesis and carcinogenesis inhibitors.
- In measurements of radical scavenging activity, reaction with the diphenylpicrylhydrazyl (DPPH) radical is used.
- Correlation between antioxidant capacity and contribution of individual phytochemicals.



Extraction by conventional solvent extraction method


Plant material
Organic solvent/ water/ CO_2
Ultrasound assisted solvent extraction (UASE)
Microwave assisted solvent extraction (MASE)
Supercritical fluid extraction (SFE)

Extraction by green extraction techniques